

Claims

✓ 1. A method for activating a nucleus from a human fetal cell comprising the steps of:

- a) separating said nucleus from its surrounding cytoskeleton to form a pretreated nucleus, and  
b) contacting said pretreated nucleus with an activating egg extract to activate said pretreated nucleus.

2. The method of claim 1, wherein said fetal cell is selected from the group consisting of keratinocyte, trophoblast, erythrocyte and leukocyte.

3. The method of claim 2, wherein said leukocyte is selected from the group consisting of neutrophil, basophile, eosinophil, and granulocyte.

4. The method of claim 1, wherein said separating is carried out using a protease and a non-ionic detergent.

5. The method of claim 4, wherein said protease is trypsin and said detergent is lysolecithin.

6. The method of claim 1, further comprising contacting said pretreated nucleus with CSF extract.

7. The method of claim 6, wherein said nucleus is activated under conditions where the nucleus and its chromosomes do not divide.

8. The method of claim 7, wherein said conditions comprise adding nocodazole to said activating egg extract or said CSF extract.

9. The method of claim 8, wherein said nocodazole is in amount less than 5  $\mu$ g/ml.

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10. The method of claim 1, wherein said nucleus is activated under conditions not suitable for nucleic acid synthesis.

5 11. A method of causing a non-dividing human nucleus to activate, using activating egg extract and CSF extract prepared from hardened eggs comprising:

- a) incubating said non-dividing human nucleus with said CSF extract prepared from hardened eggs to form a pretreated nucleus, and
- 10 b) contacting said pretreated nucleus with said activating egg extract wherein said activating egg extract is prepared from synchronously activated hardened eggs.

12. The method of claim 11, wherein said CSF extract is frozen and thawed before use.

15 13. The method of claim 11, wherein said activating egg extract is frozen and thawed before use.

14. The method of claim 11, wherein said incubating is performed using a warm-then-cold regime comprising incubating at about 25°C for at least 30 minutes

20 followed by incubation at about 4°C for at least 30 minutes.

15. The method of claim 11, wherein said incubating is performed using a warm regime at about 25°C for at least 30 minutes.

25 16. The method of claim 11, wherein  $\text{Ca}^{2+}$  is provided in said incubating step.

17. The method of claim 16, wherein said  $\text{Ca}^{2+}$  is provided in an amount greater than 100  $\mu\text{M}$ .

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18. A method for preparing an activating egg extract, comprising the steps of hardening a plurality of eggs, simultaneously inducing said eggs; and preparing an activating egg extract from said eggs wherein said eggs  
5 are induced for a length of time such that they have at least 70% of maximal activation DNA synthesis activity.

19. The method of claim 18, wherein said activating egg extract is prepared from a eukaryotic cell.

20. The method of claim 19 wherein said  
10 eukaryotic cell is an amphibian, yeast, human, echinoderm, mollusc, fish, or chicken cell.

21. The method of claim 20, wherein said eukaryotic cell is a *Xenopus* cell.

22. The method of claim 21, wherein said eggs  
15 are obtained from *Xenopus* and said length of time is greater than 10 minutes.

23. The method of claim 22, wherein said length of time is between 25 and 30 minutes.

24. A method of causing a non-dividing cell  
20 nucleus to swell comprising the steps of:

- a) separating said nucleus from its surrounding cytoskeleton to form a pretreated nucleus; and
- b) contacting said pretreated nucleus with a CSF  
25 extract supplemented with an aqueous solution or a protein kinase inhibitor.

25. The method of claim 24, wherein said nucleus is a human nucleus.

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26. The method of claim 25, wherein said CSF extract is a partially purified CSF extract supplemented with an aqueous solution and a protein kinase inhibitor.

27. The method of claim 26, wherein said aqueous solution is an appropriate buffer.

28. The method of claim 27, wherein said protein kinase inhibitor is either 6-dimethylaminopurine or staurosporine.

29. The method of claim 28, wherein said appropriate buffer is provided in an amount to dilute said CSF extract by 25% to 75%.

30. The method of claim 27, further comprising the step of adding an agent to further swell or decondense said nuclei after said step (b).

31. A method of causing chromosome formation in a non-dividing cell nucleus comprising the steps of:

- a) separating said nucleus from its surrounding cytoskeleton to form a pretreated nucleus; and
- b) contacting said pretreated nucleus with a CSF extract supplemented with a cyclin.

32. The method of claim 31, wherein said cyclin is cyclin-D90.

33. A method for activating or studying a mammalian sperm cell nucleus comprising the steps of:

- a) pretreating said sperm cell nucleus to form a pretreated sperm nucleus wherein said pretreating comprises (i) separating said nucleus from its surrounding cytoskeleton by permeabilizing said cell nuclear membrane and incubating in the presence of a protease and

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b) activating said pretreated sperm cell.

34. The method of claim 33 wherein said sperm  
5 cell nucleus is a human sperm cell nucleus.

~~35. The method of claim 34, wherein said activating is carried out by contact with a CSF extract, wherein said CSF extract is supplemented to induce nuclear swelling.~~

10                    36. The method of claim 34, wherein said  
activating is carried out by contact with a CSF extract,  
wherein said CSF extract is supplemented to induce  
chromosome formation.

37. The method of claim 34, wherein said  
15 activating is carried out using an activating extract.

38. The method of claim 33, wherein said sperm cell nucleus is from a transgenic animal.

39. The method of claim 34, wherein said human sperm cell nucleus is obtained from either a person infected with a virus or a person not infected with said virus.

40. The method of claim 39 wherein, said virus is HIV.

41. The method of claim 34, further comprises  
25 the step of analyzing said nucleus by in situ  
hybridization.

42. The method of claim 34, wherein said step (a)(i) is carried out using a detergent and trypsin.

44. The method of claim 34, wherein a CSF extract further pretreatment step is carried out between said step (a) and said step (b), comprising incubating the pretreated nuclei in CSF extract.

fertility comprising:

- a) pretreating a sperm nucleus to separate  
10 cytoskeletal protein from nucleic acid,
- b) activating said sperm nucleus,
- c) measuring activation of said nucleus  
activated in step (b).

47. The activation assay of claim 46, wherein said sperm nucleus is a human sperm nucleus.

49. The method of claim 47, wherein said activating is carried out using an activating egg extract.

steps of: 51. A viral integration assay comprising the



a) pretreating a cell nucleus to separate the nucleus from its surrounding cytoskeleton to form a pretreated nucleus,

5 b) activating said nucleus and incubating with a viral integration complex containing viral nucleic acid, wherein said integration complex is added before or after said incubating; and

c) measuring integration of viral nucleic acid into nucleic acid of said cell nucleus.

10 52. The assay of claim 51, wherein said viral nucleic acid is from HIV.

53. The assay of claim 52, further comprising a step, between said step b) and said step c), of adding an  
15 agent which inhibits integration of said HIV.

54. A viral integration assay comprising the steps of:

a) constructing a pseudonucleus from a defined DNA template,

20 b) activating said nucleus and incubating with a viral integration complex containing viral nucleic acid, wherein said integration complex is added before or after said incubating; and

c) measuring integration of viral nucleic acid  
25 into nucleic acid of said cell nucleus.

55. A product for preparing a non-dividing nucleus to activate upon subsequent treatment with activating egg extract, comprising a CSF extract prepared using an eukaryotic cell, wherein said CSF extract is  
30 supplemented with  $\text{Ca}^{2+}$ .

56. The product according to claim 55, wherein said  $\text{Ca}^{2+}$  is provided in an amount greater than 100  $\mu\text{M}$ .

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57. A product for causing nuclear swelling comprising CSF extract supplemented with a protein kinase inhibitor and/or an aqueous solution.

58. The product of claim 57, wherein said CSF extract is a partially purified extract.

59. The product of claim 58, wherein said aqueous solution is an appropriate buffer.

60. The product according to claim 59, wherein said supplement is said protein kinase inhibitor and said appropriate buffer.

61. The product according to claim 60, wherein said protease inhibitor is either 6-dimethylaminopurine or staurosporine.

62. A product for causing chromosome formation in a cell nucleus comprising a CSF extract supplemented with a cyclin.

63. The product according to claim 62, wherein said cyclin is cyclin- $\Delta 90$ .

64. A product for activating a non-dividing nucleus comprising an activating egg extract having at least 70% optimal activation activity.

65. The product of claim 64, wherein said activating egg extract is prepared from one or more eukaryotic egg.

66. The product of claim 64, wherein said activating egg extract is prepared from a plurality of *Xenopus* eggs synchronously induced for more than 10 minutes.

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67. The product of claim 66, wherein said *Xenopus* eggs are synchronously induced for 25 to 30 minutes.

68. The product of claim 66, wherein said activating egg extract is supplemented with cAMP.

69. The product of claim 66, wherein said activating egg extract is supplemented with a phosphodiesterase inhibitor.

70. A kit for activating a non-dividing nucleus comprising a first product comprising frozen activating egg extract having at least 70% optimal activation activity and a second product comprising a frozen CSF extract.

71. The kit according to claim 70, wherein said second product is supplemented with  $Ca^{2+}$ .

72. The kit according to claim 70, wherein said first product and said second product are prepared from hardened eggs.

73. The kit according to claims 70, further comprising a microchamber microscope slide.

74. A microscope slide comprising;

a) an upper surface,

b) a water repellent means having a defined thickness located upon said upper surface to define a microchamber connected by a channel to at least one well on said upper surface, and

c) said microchamber shaped to enhance flushing of said microchamber.

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75. The microscope slide of claim 74, wherein said microchamber is in a teardrop-shape or a pear-shape.

76. The microscope slide of claim 74, wherein two wells are provided at opposite ends of said microchamber and each of said wells are connected to said microchamber by a channel.

77. The microscope slide of claim 74, wherein said microchamber has a defined volume between 5 and 50  $\mu$ l.

10 78. The microscope slide of claim 74, wherein said microchamber has defined volume between 10 and 20  $\mu$ l.

79. The microscope slide of claim 74, wherein said water repellent means is a tape or a coating on said upper surface of said slide.

15 80. The microscope slide of claim 74, wherein said water repellent means is a TEFLON® coating.

81. The microscope slide of claim 74, wherein said water repellent means is a plastic containing tape.

20 82. The microscope slide of claim 74, wherein said upper surface is treated to enhance cell growth compared to an untreated slide.

83. The microscope slide of claim 74, wherein said microscope slide is sterile.

25 84. The microscope slide of claim 74, wherein said microchamber or said at least one well contains an antibody to a human fetal cell.

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85. A method for analysis of growth or manipulation of a cell or cell component comprising providing a microscope slide of claim 74 and placing said cell or said cell component in said microchamber.

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86. The method of claims 74, wherein a fluid is introduced into one said well and is allowed to enter said microchamber and then is removed from said microchamber.

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